

RESEARCH ARTICLE

Toxicity Effect of *Bougainvillea glabra* (Paper Flower) Water Extracts on Zebrafish Embryo

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Abstract: Background: *Bougainvillea glabra*, or paper flower, is an ornamental plant known to possess various pharmacological activities. However, there is little information on the potential toxicity of this plant. Therefore, the aim of this study was to assess the acute toxicity and the potential teratogenic activity of water extracts of *B. glabra* bracts on zebrafish embryos which were selected as the model in this study.

Methods: The pink, purple, and dark pink bracts of *B. glabra* were extracted using the Soxhlet method. Embryos with the same division stage were selected and treated with the extracts (1-300 µg/mL) for 72 h. The mortality of the embryos was recorded and the teratogenicity effect induced by the extracts was identified. The yolk sac area and pigmentation were quantified using Image-J. The data were analyzed by IBM SPSS 22.0.

Results: All extracts within the tested concentrations did not induce death in embryo, except the pink bract extract with LC₅₀ of 85.51 µg/mL. In the teratogenicity study, all the extract-treated embryos showed yolk sac edema at different concentrations, and the defect observed was independent of the concentrations. On top of that, the purple bract extract induced hypopigmentation in embryo, significantly at 30 µg/mL compared to control.

Conclusion: This study concluded that the water extracts derived from the pink, purple, and dark pink bracts of *B. glabra* have mild toxicity toward embryo.

Keywords: Zebrafish embryos; *Bougainvillea glabra*; Bracts; Acute toxicity

1. INTRODUCTION

Bougainvillea glabra is a type of flower that belongs to the Nyctaginaceae family. It is commonly known as paper flower and their bracts display multiple colors such as pink, purple orange, white and so on. True flowers are a small, white or yellow cluster of three flowers in the center surrounded by bracts. Other than being an ornamental plant at roadside, garden, or household, *B. glabra* also possesses medicinal properties, which are generally used by folks to treat diarrhea, ulcer, sore throat, cough, tumor, as well as lower blood pressure [1,2]. In addition, it has also been reported for its uses

in regulating menstruation and vaginal discharge, treatment of hepatitis, as well as for its roles as the antioxidant, anti-inflammatory [3], and antimicrobial agents [4,5]. However, an understanding of the acute toxicity of *B. glabra*, especially on the vertebrate, is still warranted.

The use of the higher experimental animal models in evaluating the toxicity and safety of new compounds has always been criticized and concerned by the ethic committees of their respective institutions around the world. Zebrafish (*Danio rerio*) are an ideal model that connects the research gap between *in vitro* cell line assays and *in vivo* higher animal models [6]. The high degree of homology

in the genes (70%) shared by the zebrafish and human is the reason why the zebrafish embryos are widely used in the study of carcinogenesis [7], embryogenesis on toxicant exposure [8], angiogenesis [9], diabetes, cardiovascular, and neurodegenerative diseases [10]. Furthermore, zebrafish have high fecundity which enables high-throughput assays and high transparency of the embryos which allows visualization of the drug effects on internal organs [11]. This study was sought to investigate the acute toxicity and teratogenic effect of water extracts derived from the purple, pink, and dark pink bracts of *B. glabra*, using zebrafish embryo as a model.

2. MATERIALS AND METHODS

2.1. Collection of *B. glabra*

The bracts of *B. glabra* were collected from the University Botanical Garden at Universiti Putra Malaysia (Serdang, Selangor, Malaysia). The plants were identified and authenticated by a botanist from the Institute of Bioscience, Universiti Putra Malaysia (Plant Identification Number: SK3206/17).

2.2. Extraction

The bracts were isolated and were then air-dried for 1 week at room temperature. The dried plant materials were pulverized using an electrical grinder. The powdered materials were deflated using petroleum ether, ethanol, and water in a successive manner using the Soxhlet apparatus. The water extracts were then freeze-dried and stored at 4°C until further use.

2.3. Zebrafish Husbandry

Wild-type zebrafish were purchased from Danio Assay Laboratories Sdn Bhd, Malaysia. The male and female zebrafish were maintained in different tanks. The temperature of the fish tanks was maintained at 25°C on a fixed 11 h photoperiod per day. Healthy adult male and female zebrafish were chosen and placed in the mating chamber overnight for 13 h in the dark. On the next day, the divider was removed and the eggs were collected after spawning. The fertilized chorionated embryos that were uniformly divided at 16-cell stage were selected and placed in the 1 × E3 embryo media (5.0 mM NaCl, 0.17 mM KCl, 0.33 mM CaCl₂, and 0.33 mM MgSO₄). Ten embryos were placed in each well of 24-well plates with E3 media, and *B. glabra* extracts of different concentrations were then added into each well and incubated for 72 h (until hatching) at room temperature. In this study, chorionated embryos were used to avoid high mortality and dysmorphology as a result of the chorion removal procedure, and developmental defects were compared and normalized with the negative control (untreated, only E3 media). The experiment was considered valid if the control groups did not show more than 10% of lethality and defect on the 3rd day after fertilization [12]. The extract concentrations used were based on the Organization for Economic Co-operation and Development guideline [13]. The experiment was performed

according to the protocol approved by the Institutional Animal Care and Use Committee, Management and Science University (Approval Code: AE-MSU-022).

2.4. Statistical Analysis

All data were compared and analyzed using the Mann–Whitney U-test (IBM SPSS version 22). LC₅₀ was calculated using the SPSS probit analysis. $P < 0.05$ is considered statistically significant.

3. RESULTS

The acute toxicity of water extracts derived from the pink, purple, and dark pink bracts of *B. glabra* was determined based on the coagulation of embryo and lack of somite formation for every 24 h. Ten embryos were treated with the water extracts derived from the pink, purple, and dark pink bracts of *B. glabra* at different concentrations (0.3, 1, 3, 10, 30, 100, and 300 µg/mL). Purple and dark pink bract extracts were non-toxic to the embryos at the tested concentrations, while pink bract extract was found to be toxic to embryo with lethal concentration 50% (LC₅₀) of 85.51 µg/mL (**Fig. 1**). Overall, no delay in the hatching rate and growth retardation as a result of these extracts was observed.

For teratogenicity study, two major phenotypic defects which were the yolk sac edema and hypopigmentation were observed in embryos treated with *B. glabra* water extracts. To score the yolk sac edema, treated embryos were examined under a stereomicroscope. As shown in **Fig. 2**, all the extracts induced yolk sac enlargement independently of the concentrations. The yolk sac area was quantified in every embryo (**Fig. 2A**). The highest induction was observed in the zebrafish embryos exposed to the dark pink bract extract at concentrations of 1 µg/mL, 3 µg/mL, and 300 µg/mL, and the enlargement was about 15–20% compared to untreated control. The purple bract extract induced approximately 11% yolk sac enlargement at 300 µg/mL, whereas pink bract extract induced about 16% at the concentration of 30 µg/mL, compared to untreated control (**Fig. 2B**).

The treatment with water extract from purple bract of *B. glabra* caused hypopigmentation in the zebrafish embryos

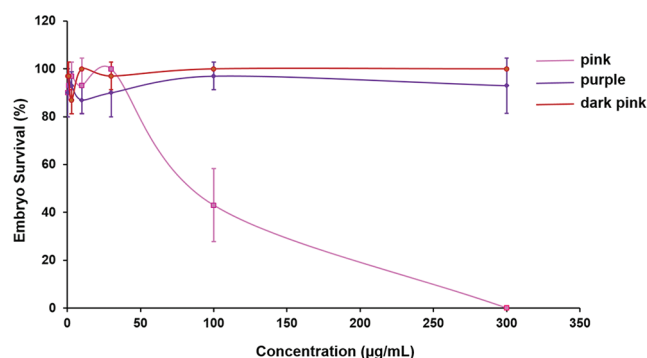


Fig. (1). Toxicity profile of water extracts derived from the pink, purple, and dark pink bracts of *B. glabra* in zebrafish embryos. Data represent mean \pm SD of three independent experiments.

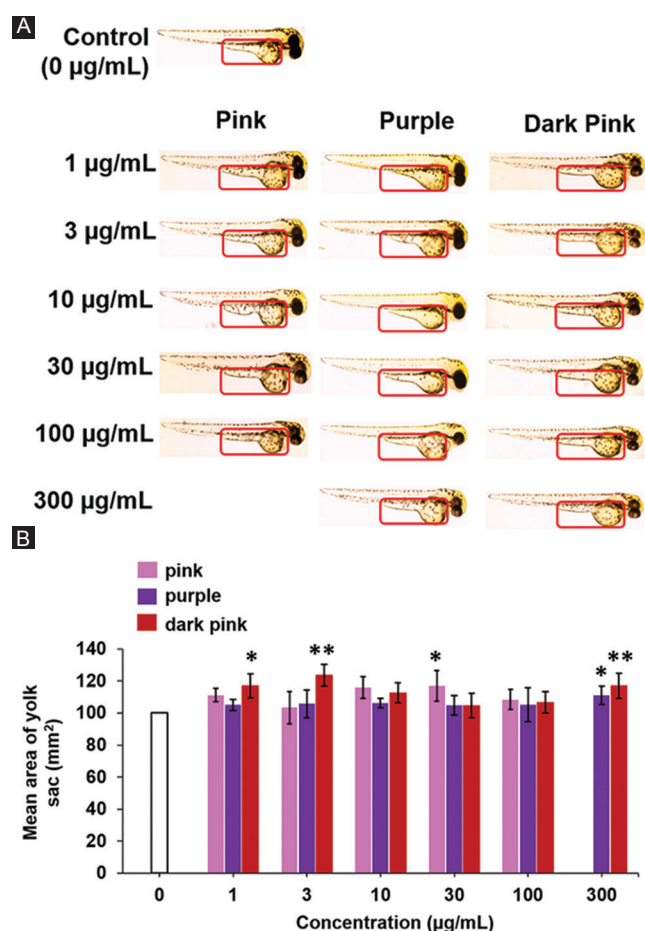


Fig. (2). Yolk sac area of zebrafish embryos treated with water extracts derived from the pink, purple, and dark pink bracts of *Bougainvillea glabra*. (A) Yolk sac area was quantified using Image-J. (B) Mean \pm SEM area of yolk sac of three independent experiments. * $P < 0.05$, ** $P < 0.005$ versus control (0 $\mu\text{g/mL}$).

(Fig. 3A), in which the reduction was significant only at 30 $\mu\text{g/mL}$ and 300 $\mu\text{g/mL}$, which was approximately 15% (Fig. 3B). Conversely, no significant hypopigmentation effect was observed in embryo treated with both pink and dark pink bract extracts at all concentrations.

4. DISCUSSION

Bougainvillea species is consumed in the form of decoction as traditional medicine in some Asian communities to treat cough, diarrhea, hepatitis, and sore throat and is used as an anti-viral, anti-inflammatory, and antibacterial agent [1]. Most of the reported studies focused on the leave extracts and mainly on the non-polar and mid-polar secondary metabolites and their biological activities [1,3], but the study on the flowers and bracts is limited. Hence, this research was sought to explore the toxicity of water extracts derived from the pink, purple, and dark pink *B. glabra* bracts and the associated water-soluble compounds that have not been thoroughly studied thus far, using zebrafish embryo. The preparation of water extracts in this study, henceforth, simulates the traditional practice for preparing the flower for medicinal purposes. Based on the data obtained, the water extracts of purple and dark pink

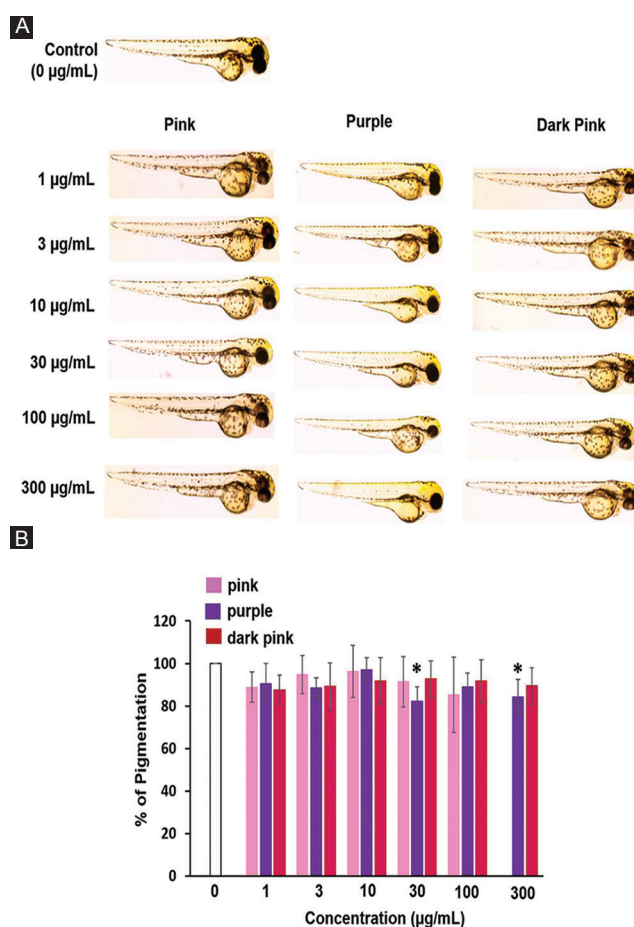


Fig. (3). Pigmentation of zebrafish embryos treated with water extracts derived from the pink, purple, and dark pink bracts of *Bougainvillea glabra*. (A) Percentage of pigmentation was quantified from the yolk sac region using Image-J. (B) Mean \pm SEM pigmentation of three independent experiments. * $P < 0.05$ versus control (0 $\mu\text{g/mL}$).

B. glabra bract were non-toxic to the embryo, except pink with LC_{50} of 85.51 $\mu\text{g/mL}$. When compared with the methanolic extracts of *Bougainvillea* flower of five different colors (white, orange, pink, red, and purple) [4], water extract of pink bract is more toxic than the methanolic extracts, but not for purple. As reported by Ali *et al.*, pink flower methanolic extract was non-toxic to brine shrimp up to 1 mg/mL, whereas the LD_{50} of the purple bract extracts ranged from 0.1 to 1 mg/mL [4]. The discrepancy in the toxicity level between the two studies might be due to the differences in the extraction method, particularly the extraction solvent which may yield phytochemicals with varying characteristics [2]. Moreover, a study on the water extract of *B. glabra* leaves revealed that the extract was non-toxic, up to a dose of 2000 mg/kg when given orally to mice [2]. This further warrants studies on the safety of *B. glabra* water extracts. Nonetheless, the oral administration of ethanolic flower extract of *B.x buttiana* was also reported to be safe to mice at the dose of 1600 mg/kg since no toxicity and behavioral changes were observed [14].

Yolk sac enlargement and hypopigmentation defects are the major phenotypic defects observed in the extracts treated embryos. The yolk sac edema observed might be due to

the effects of the hemostasis, impaired blood flow or fluid imbalance. This postulation is supported by a study claiming the impaired blood flow and fluid imbalance is related to pericardial and yolk sac edema in zebrafish [15]. A study by Mishra and Tandon demonstrated that the water extracts derived from the leaves of a close species, *B. spectabilis* affected the hematologic cell count and packed cell volume in mice [16], which further suggests the possibility of *Bougainvillea* species in affecting the circulatory system. However, pericardial edema was absent in this study and the yolk sac edema induced was approximately 10-20%, suggesting that the toxic effect induced by *B. glabra* extracts in the zebrafish embryos is mild.

In anticancer drug discovery, zebrafish phenotypic assay is applied for the rapid screening of bioactive compounds with anticancer properties due to the high degree of homology between human carcinogenesis and zebrafish embryogenesis [7,12,17]. The reason of hypopigmentation observed in the embryo treated with the purple bract extract is unknown. However, hypopigmentation caused by compound treatment in zebrafish embryo was reported to be associated with the inhibition of mammalian target of rapamycin (mTOR) expression, which is essential for the pigment formation in zebrafish [7]. The mTOR expression is highly elevated in rapidly proliferating cancer cells. This suggests that water extract from purple bract might have bioactive substances that can specifically interfere with the mTOR expression, leading to hypopigmentation. The high antioxidative properties of *Bougainvillea* spp. [1,5], as well as teratogenicity induced in the zebrafish embryo, altogether suggest that *Bougainvillea* species might possess anticancer properties. Based on our findings, further studies to explore the potent anticancer activity of *Bougainvillea* spp., using cellular and molecular assays, are warranted.

5. CONCLUSION

The water extracts derived from the purple and dark pink *B. glabra* bracts are non-toxic and can be tolerated by the embryos though in some cases, the embryos showed minor phenotypic alterations such as mild yolk sac edema and hypopigmentation.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interest.

AUTHOR CONTRIBUTIONS

L.E.T. performed the experiments on the zebrafish embryo and analyzed the data.

C.H.N. performed the extraction of bract.

C.S.K. and B.F.L. designed the experiments and wrote the paper.

All authors read and approved the final manuscript.

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